APPENDIX A
U.S. Serial No. 09/513,575

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#26

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
John Harrington, *et al.*

Serial No.: 09/~~479,122~~ 513,997

Filed: January 7, 2000

For: **COMPOSITIONS AND METHODS FOR NON-TARGETED ACTIVATION OF ENDOGENOUS GENES**

Group Art Unit: 1632

Examiner: Brunovskis, P.

Attorney Docket No.: 0221-0003C

Commissioner of Patents
Washington, D.C. 20231

~~OK to Enter~~

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

The undersigned, John J. Harrington, declares and states:

1. I am an inventor of the above-captioned patent application, U.S. Application No. 09/479,122, filed January 7, 2000, entitled "Compositions and Methods for Non-Targeted Activation of Endogenous Genes." I am the subject of the attached Curriculum Vitae and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am an expert in the field of non-homologous recombination, eukaryotic gene expression and gene cloning and am qualified to speak on the skill and knowledge of the person of ordinary skill in these fields.

Match and Return

2. I have read and understand the subject matter of the above-captioned application. I have read and understand the Office Action dated March 15, 2001, rejecting claims 75, 81 and 83–88 under 35 U.S.C. § 112, first paragraph, on the grounds that it would have required an undue burden of experimentation to practice the claimed invention. It is my opinion, based on the scientific evidence and reasoning below, that the methods that are the subject of the rejected claims could have been made and used by the person of ordinary skill in the art, as of the filing date of September 26, 1997 (the earliest effective filing date), by routine and ordinary experimentation, using the Applicants' specification and general knowledge in the art as a guide.

3. It is my opinion, based on the scientific evidence and reasoning set forth below, that the methods that are the subject of the rejected claims provided a practical, real-world use. I assert this because the methods provide a way to use cells to produce protein in an animal and using cells to produce protein in an animal had a practical, real-world use as of the Applicants' earliest effective filing date.

4. It is my further opinion that the person of ordinary skill in the art reading Applicants' specification would have immediately appreciated that producing protein from Applicants' cells in an animal was useful, as of the Applicants' earliest effective filing date. I assert this because cell-based protein production in an animal was known by the art to be useful and the claimed methods were disclosed as a way to provide cell-based protein production in an animal.

5. As a rationale for the rejection, the Examiner has stated that the specification discloses only one real-world use for the claimed method: cell therapy. I do not agree with this statement because the specification discloses cell-based protein production in an animal and there was real-world use for cell-based protein production in various contexts besides cell therapy. Some of these will be discussed further below in this Declaration. In addition, the specification also discloses the isolation and purification of protein produced in an animal by the cells of the invention. This disclosure clearly demonstrates a utility distinct from cell therapy, since cell therapy does not involve subsequent purification of the protein following expression *in vivo*. Isolation and purification of proteins produced in an animal had real-world use. Thus, the specification does disclose a process with real-world use in addition to use for cell therapy.

The Examiner has also asserted that he is unaware of any well-established utility for the claimed method except cell therapy. I, therefore, point out that there was well-established utility for the claimed method for uses in addition to cell therapy.

I understand a well-established utility to be a real-world use that would have been immediately apparent to the person of ordinary skill in the art reading the Applicants' specification. It is my opinion that both non-therapeutic, cell-based protein production in an animal and isolation of proteins produced from cells placed in an animal would have been recognized immediately as having practical use by the person of ordinary skill in the art.

6. My opinions and conclusions in this Declaration are supported by evidence in the form of scientific references that I will discuss in the paragraphs that follow. These references show that there were non-therapeutic uses for introducing a cell into an animal to produce a desired protein from the cell. The Applicants' disclosure directs the artisan to introduce Applicants' cells into an animal to produce protein from the cell. Having access to the references, the person of ordinary skill in the art would have readily appreciated that the claimed methods also had non-therapeutic utility.

7. The following references, available as of the earliest effective filing date, show some non-therapeutic practical uses for producing protein from cells introduced into an animal.

8. Brodeur et al., Kints et al., and Stewart et al. demonstrate the utility of expressing a protein from a cell introduced into an animal. The utility is not based on cell therapy. Specifically, each of these authors describes a method for introducing hybridomas into mice or rats to produce large quantities of antibodies. The antibodies are produced from endogenous antibody genes in the hybridomas. The purpose of this work was to optimize conditions for producing antibodies so that the antibodies could be purified. It is stated in these references that there are advantages to producing antibodies *in vivo*. The references thus demonstrates a utility for protein production *in vivo* that does not involve cell-based therapy.

The Applicants' specification discloses and claims methods for expressing desired protein from endogenous genes in eukaryotic host cells and subsequently introducing the cells

into an animal to produce protein *in vivo*. Applicants' specification, in fact, also discloses using hybridomas to express endogenous genes using the methods of the invention. See U.S. Application No. 08/941,223, page 30, line 22 and U.S. Application No. 09/276,820, page 53, line 29. The specification also discloses expression of antibodies using Applicants' methods. See U.S. Application No. 08/941,223, page 22, line 25 and U.S. Application No. 09/276,820, page 43, line 16. The specification also discloses isolating and purifying proteins expressed *in vivo*.

Based on these similarities, it is my opinion that it would have been readily apparent to someone skilled in the art that Applicants' cell lines and hybridomas, expressing endogenous antibodies, could be used to produce antibodies *in vivo*. It is, therefore, my opinion that there was practical, well-established utility for using the claimed method.

9. U.S. Patent No. 5,733,761 describes uses for production of proteins *in vivo*. The uses are not based on cell therapy. Desired endogenous proteins are activated by homologous recombination. In column 3, starting at line 17, it is indicated that the cells introduced into the animal are useful for eliciting antibody production or for immunizing humans or animals against pathogenic agents. The antigens can be used to produce antibodies that are then used for therapeutic or diagnostic purposes. This U.S. patent is a continuation of U.S. Application No. 07/985,586. Accordingly, it contains the same specification. WO 94/12650 is a PCT application claiming priority to U.S. Application No. 07/985,586. The relevant text in the U.S. application, discussed above, is found in the PCT application on page 5. The PCT application was published

in June 1994. Accordingly, this information was available to the person of ordinary skill in the art well before the earliest effective filing date of Applicants' claims.

The patent specification discloses non-therapeutic uses in addition to those discussed directly above. In column 14, starting at line 1, the disclosure discusses implanting cells for agricultural use, for example, meat and dairy production. It is my opinion that this disclosure would have been recognized by the person of ordinary skill in the art to include such uses as the delivery of hormones to an animal through the implanted cells. Line 6 then goes on to discuss the implanted cells as also being useful for eliciting antibody production for immunizing humans and animals against pathogenic agents or for producing antibodies useful for therapeutic and diagnostic purposes. This discussion can be found in the corresponding PCT application on pages 27 and 28.

U.S. Patent No. 5,641,670 has a corresponding PCT application, WO 95/31560, published November 23, 1995. In the U.S. patent, column 3, starting at line 27, the specification discusses the use of homologously recombinant cells to immunize animals or produce antibodies in immunized animals. The patent specification also discusses agricultural uses, citing the production of bovine growth hormone for dairy production. See column 4, lines 37–39. Starting at line 47, the specification also discusses *in vivo* immunization and use of the cells to produce antibodies for diagnostic and therapeutic purposes. In column 18, line 40, the disclosure discusses implanting the cells for agricultural uses (i.e., meat and dairy production).

Accordingly, both U.S. Patent Nos. 5,641,670 and 5,733,761, and their corresponding PCT applications, demonstrate utility for production of protein *in vivo* that is not based on cell therapy. It is my opinion that it would have been readily apparent to the person of ordinary skill in the art that the protein-producing cells disclosed in Applicants' specification had the same non-therapeutic utility. It is my opinion, therefore, that the claimed method had practical, well-established utility.

10. Shaw et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell-based therapy. The reference describes the introduction of cells expressing IL-4 and IL-10 into a mouse. The purpose of this work was to produce IL-4 and IL-10 protein *in vivo*. Another purpose of this work was to test the biological activity of the proteins in a disease model. Finally, the authors state that *in vivo* production of protein from introduced cells allows them to produce the proteins at a specific site in the animal, as opposed to systemic delivery of an injected protein.

The Applicants' application also describes introducing cells expressing a protein of interest into an animal to produce protein. The Applicants' specification describes activation and expression of a variety of proteins, including IL-4 and IL-10. See U.S. Application No. 08/941,223, page 22, line 30; page 23, line 10; and page 48, line 16 and U.S. Application No. 09/276,820, page 43, lines 21–23.

Based on these similarities, it is my opinion that using the claimed method to produce IL-4 and IL-10 *in vivo* would have been readily apparent. It would also have been apparent from this reference that producing protein in an animal by the present method is useful to test the biological activity of the protein *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

11. Chen et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. Specifically, the reference describes a method for introducing the nerve growth factor gene into normal fibroblasts, and subsequently introducing the cells expressing NGF into the nucleus basalis magnocellularis (i.e. a region of the brain) of rats. Following implantation, the rats were tested using a Morris water maze to assess their spatial memory ability. The authors show that expression of NGF *in vivo* can reverse naturally occurring age-related memory loss. The authors state that production of protein in an animal "can be used both to explore basic biological questions concerning the structure and function of the brain or as a form of somatic gene therapy. A principal advantage of this approach is the local intraparenchymal delivery of factors to responsive cells, which allow one to examine the effects of the factors on specific populations of cells. Additionally, following the implantation of the transfected cells there is no need for any further invasive procedure, such as the chronic infusion of various factors into the cerebral ventricles by osmotic minipump." Thus, it was appreciated that *in vivo* expression of a protein is useful to define a biological process and potentially as a therapeutic. This art also appreciated the advantages of expressing a protein *in vivo*, as opposed to introducing the purified protein into the animal.

Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce the protein. It also describes activation and expression of a variety of proteins, including nerve growth factors. See U.S. Application No. 08/941,223, page 23, line 6; page 25, line 24; and page 48, line 22 and U.S. Application No. 09/276,820, page 43, line 28. It further describes the use of primary cells and fibroblasts to express a protein of interest. For disclosure of primary cells, see U.S. Application No. 08/941,223, page 30, lines 2 and 13–15 and U.S. Application No. 09/276,820, page 53, lines 9 and 20–22. For fibroblasts, see U.S. Application No. 08/941,223, page 30, lines 7 and 21 and U.S. Application No. 09/276,820, page 54, line 15. It also describes use of any eukaryotic cell, including rat cells. For rat cells, see U.S. Application No. 09/276,820, page 10, line 5 and page 54, line 2.

Based on these similarities, it is my opinion that using the claimed method to produce nerve growth factor *in vivo* would have been readily apparent. Furthermore, it is my opinion that a person of skill in the art would have appreciated the utility of *in vivo* protein expression using the present method to study biological processes including memory and cognition. It is my opinion, therefore, that the claimed invention had practical, well-established utility.

12. Garver et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. Specifically, the reference describes a method for introducing the human alpha 1-antitrypsin (alpha 1AT) gene into normal mouse fibroblasts, and subsequently

introducing the cells expressing alpha 1AT into the peritoneal cavities of mice. The authors show that human alpha 1AT could be detected in the sera and epithelial surface of the lungs, and that upon recovery, the mouse fibroblasts continued to express alpha 1AT four weeks following introduction into the animal. The authors state that production of protein in an animal is useful as a model for gene therapy and as an "approach to study the *in vivo* effects of such hormones and growth factors."

Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce protein. It also describes activation and expression of a variety of proteins, including alpha 1AT. See U.S. Application No. 08/941,223, page 22, line 24; page 23, line 3; and page 48, line 19 and U.S. Application No. 09/276,820, page 43, line 26. It also describes the use of primary cells and fibroblasts to express a protein of interest. It describes use of any eukaryotic cell, including mouse cells. See U.S. Application No. 09/276,820, page 10, line 5 and page 54, line 2.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the Applicant's method to produce alpha 1AT *in vivo*. Furthermore, it is my opinion that a person of skill in the art would have appreciated the utility of *in vivo* protein expression using the present method to "study the *in vivo* effects of such hormones and growth factors," or other genes. It is my opinion, therefore, that the claimed method had practical, well-established utility.

13. McNiece et al. demonstrates the utility of producing protein from a cell placed in an animal for other than cell therapy. This reference reports the introduction of a cell line, WEHI-3, into mice to produce large amounts of IL-3. IL-3 was expressed from the endogenous IL-3 gene in the cell line. Following introduction into the animal, IL-3 activity was detected in both the sera and ascites fluids of the mice. The IL-3 protein was subsequently purified. In the last paragraph, page 1074, the reference states "The WEHI-3 tumor-bearing mice may thus provide a model for the study of the effects *in vivo* of SF and IL-3 on bone marrow cells." The reference thus demonstrates a utility for protein production *in vivo* other than for cell-based therapy.

Applicants' specification discloses introducing cells expressing a protein of interest into an animal to produce protein. The protein can optionally be purified. It also describes activation and expression of a variety of proteins, including cytokines, and specifically interleukins. For interleukins, see above. For cytokines, see U.S. Application No. 08/941,223, page 22, line 24 and U.S. Application No. 09/276,820, page 43, line 15. It also describes the use of a variety of cell lines similar to WEHI-3.

Based on these similarities, it is my opinion that a person of ordinary skill in the art would have recognized that Applicants' method could be used to produce IL-3 *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

14. Ishihara et al. demonstrates the utility of expressing a protein from a cell placed in an animal for other than cell therapy. The reference describes the *in vivo* production of a protease by introducing a tumor cell line, AH109A, into rats. The protease was produced from the endogenous protease gene in the tumor cell line. The purpose of the experiment was to assess the effect of protease expression on tumor cell invasiveness. The protease was also isolated from serum protein and purified 1150-fold to assess and characterize the protease produced *in vivo*.

The Applicants' specification describes introducing cells expressing a protein of interest into an animal to produce protein. The protein can optionally be purified. It also describes activation and expression of a variety of proteins. These include, but are not limited to, proteases, including TPA, urokinase, and protein C. For TPA, see U.S. Application No. 08/941,223, page 22, line 27 and page 48, line 14 and U.S. Application No. 09/276,820, page 43, line 19. For urokinase, see U.S. Application No. 08/941,223, page 23, line 6 and page 48, line 22 and U.S. Application No. 09/276,820, page 43, line 28. For protein C, see U.S. Application No. 08/941,223, page 23, line 4 and page 48, line 20 and U.S. Application No. 09/276,820, page 43, line 27. It also describes the use of a variety of tumor cell lines. These can include a hepatoma cell line, Hep G2, similar to the hepatoma AH109A tumor cell line. For Hep G2, see U.S. Application No. 08/941,223, page 30, line 20 and U.S. Application No. 09/276,820, page 53, line 27. The specification states that cell lines useful for activating endogenous genes can be derived from any tissue. Liver cells and hepatoma cells are specifically cited. For hepatoma cell lines, see U.S. Application No. 08/941,223, page 30, line 21 and U.S. Application No.

09/276,820, page 53, line 28. For liver cells, see U.S. Application No. 08/941,223, page 30, line 4 and U.S. Application No. 09/276,820, page 53, line 11.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the claimed method to produce proteases, such as TPA, urokinase and protein C, *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

15. Bronson et al. demonstrates the utility of expressing a protein from a cell *in vivo* for other than cell therapy. The reference reports introducing the bcl-2 gene into mouse embryonic stem cells by homologous recombination. The embryonic stem cells were injected into blastocysts, and subsequently introduced into a pseudopregnant mouse using standard transgenic procedures. Bcl-2 was used as a test gene to establish the feasibility of this transgenic approach.

The Applicants' application describes introducing cells expressing a protein of interest into an animal to produce protein. The application describes activation and expression of a variety of proteins. The present invention describes the use of a variety of cell types including cells isolated from an embryo and stem cells. For embryo cells, see U.S. Application No. 08/941,223, page 30, line 6 and U.S. Application No. 09/276,820, page 53, line 13 and page 54, line 14. For stem cells, see U.S. Application No. 08/941,223, page 30, line 8 and U.S. Application No. 09/276,820, page 53, line 14 and page 54, line 16.

Based on these similarities, it is my opinion that a person of skill in the art would have recognized the utility of using the present method to produce a desired protein, as a test gene for transgenics, *in vivo*. It is my opinion, therefore, that the claimed method had practical, well-established utility.

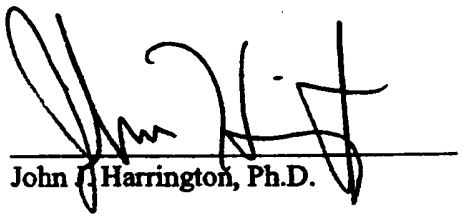
16. The cited references cumulatively show various art-known uses of cell-based expression of a desired protein in an animal besides cell-based therapy. This evidence demonstrates real-world and well-established utility for cell-based protein expression in an animal that is not cell-based therapy. Since the referenced methods were useful and would have been recognized as useful, Applicants' claimed methods also would have been useful and would have been recognized as such.

Furthermore, because *in vivo* cell-based protein production was not confined (in the literature) to one or two specific proteins or classes of protein, I believe that the person of ordinary skill in the art would have realized that *in vivo* cell-based expression could be useful for any number of proteins. This being the case, they would have also realized that Applicants' methods would also be useful for a desired protein and not just a limited class.

SUMMARY

In summary, the literature shows that cell-based protein production in an animal was useful in a variety of contexts unrelated to cell-based therapy. Therefore, non-therapeutic methods using Applicants' cells to produce protein in an animal also were useful.

The relevant literature on non-therapeutic uses for cells expressing protein in an animal was available to the person of ordinary skill in the art at the earliest effective filing date. Therefore, the person of ordinary skill in the art would have known of these uses as of this date. Accordingly, as of this date, it would have been readily apparent that Applicants' cells could be used non-therapeutically. Thus, there was a well-established non-therapeutic utility for Applicants' methods.



John J. Harrington, Ph.D.

9/5/01

Date

Credible Utility for In Vivo Protein Production

"Credible utility" is based on "whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided." Applicants respectfully submit that the utility of the production of a desired protein in an animal would have been believable to the person of ordinary skill in the art at the time that the application was filed based on the evidence and discussion in the Declaration.

Well-Established Utility

"Well-established utility" is defined as "a specific, substantial and credible utility, which is well-known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art." Utility Guidelines Training Materials, page 7. Applicants submit that the using cells non-therapeutically to produce a desired protein in an animal had well-established utility at the time of Applicants' earliest filing.

The issue of well-established utility is discussed in the attached Declaration. Scientific evidence showing well-established utility is in the form of references from the scientific literature available to the person of ordinary skill in the art at the time that Applicants' earliest application was filed. The references show that non-therapeutic *in vivo* expression of proteins from implanted cells was a developed art at the time of filing. Accordingly, the person of ordinary skill in the art would have known of the non-

therapeutic utility of protein expression from cells introduced into an animal. Applicants' specification teaches protein expression from cells introduced into an animal. Because the non-therapeutic utility of protein expression from cells introduced into an animal was known to the person of ordinary skill, the non-therapeutic utility of Applicants' methods would have been readily apparent.

Thus, the Examiner is respectfully directed to the attached Declaration for the discussion and evidence supporting these assertions. Based on the evidence in the Declaration, Applicants believe that they have met their burden of showing that the non-therapeutic use of Applicants' claimed methods was a well-established utility.

Substantial Utility Disclosed

The Examiner has also asserted that the only real-world use *disclosed* in the specification was for cell therapy. Applicants respectfully disagree. The isolation and purification of proteins produced by cells introduced into an animal *in vivo* is disclosed in Applicants' specification. These have a real-world use. References discussed in the Declaration are evidence of this use.

However, Applicants point out that even if the isolation and purification of these proteins had not been disclosed, the claimed method still would have had a well-established utility. Please see the section headed "Well-Established Utility."

Assertions of Utility in U.S. Application No. 08/941,223

Applicants' specification discloses the utility of isolating the protein produced *in vivo*. For the Examiner's convenience, the relevant text is given below.

Page 7, lines 3–9

- The cell over-expressing the gene can be cultured *in vitro* so as to produce desirable amounts of the gene product of the endogenous gene whose expression has been activated or increased. The gene product can then be isolated and purified.

Alternatively, the cell can be allowed to express the desired gene product *in vivo*.

Page 8, lines 10–17

- The invention also encompasses methods for using the cells described above to overexpress a gene that has been characterized (for example, sequenced), uncharacterized (for example, a gene whose function is known but which has not been cloned or sequenced), or a gene whose existence was, prior to over-expression, unknown. The cells can be used to provide desired amounts of a gene product *in vitro* or *in vivo*. The gene product can then be isolated and purified if desired. It can be purified by cell lysis or from the growth medium (as when the vector contains a secretion signal sequence).

Page 9, lines 4–9

- The invention accordingly is also directed to methods of using libraries of cells to overexpress endogenous genes. The

library is screened for the expression of the gene and cells are selected that express the desired gene product. The cell can then be used to purify the gene product for subsequent use. Expression in the cell can occur by culturing the cell *in vitro* or by allowing the cell to express the gene *in vivo*.

Page 13, lines 1–2

- The methods are also capable of producing over-expression of known and/or characterized genes for *in vitro* or *in vivo* protein production.

Page 16, lines 9–15

- The cell over-expressing the gene can be cultured *in vitro* so as to produce desired amounts of the gene product of the endogenous gene that has been activated or whose expression has been increased. The gene product can then be isolated and purified to use, for example, in protein therapy or drug discovery.

Alternatively, the cell expressing the desired gene product can be allowed to express the gene product *in vivo*.

Pages 16–17, lines 25–30 and 1–2

- The cell over-expressing the gene is cultured such that amplification of the endogenous gene is obtained. The cell can then be cultured *in vitro* so as to produce desired amounts of the gene product of the amplified endogenous gene that has been activated or whose expression has been increased. The gene product can then be isolated and purified.

Alternatively, following amplification, the cell can be allowed to express the endogenous gene and produce desired amounts of the gene product *in vivo*.

Page 17, lines 11–17

- The cell over-expressing the gene can be cultured *in vitro* so as to produce desirable amounts of the gene product of the endogenous gene whose expression has been activated or increased. The gene product can then be isolated and purified.

Alternatively, the cell can be allowed to express the desired gene product *in vivo*.

Page 29, lines 24–25

- Cells produced by this method can be used to produce protein *in vitro* (e.g., for use as a protein therapeutic) or *in vivo* (e.g., for use in cell therapy).

Page 35, lines 21–26

- The invention is also directed to methods of using libraries of cells to over-express an endogenous gene. The library is screened for the expression of the gene and cells are selected that express the desired gene product. The cell can then be used to purify the gene product for subsequent use. Expression of the cell can occur by culturing the cell *in vitro* or by allowing the cell to express the gene *in vivo*.

As is clear from the above text, isolation and purification of the protein produced in the animal are disclosed. The utility was substantial and also would have been readily

apparent to the person of ordinary skill in the art. See the references addressed in the Declaration that disclose isolating and purifying protein produced *in vivo*.

Accordingly, the specification asserts utilities other than cell therapy that are specific, substantial, credible, and well-established.

LOCUS HUMP58GTA 3863 bp mRNA linear PRI 27-APR-1993
DEFINITION Human p58/GTA (galactosyltransferase associated protein kinase) mRNA, complete cds.
ACCESSION M37712
VERSION M37712.1 GI:189480
KEYWORDS galactosyl transferase associated protein kinase; p58 galactosyltransferase associated protein kinase; p58/GTA protein kinase.
SOURCE Human fetal liver, cDNA to mRNA, clones hp58[2,3,4, and 5].
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 3863)
AUTHORS Bunnell,B.A., Heath,L.S., Adams,D.E., Lahti,J.M. and Kidd,V.J.
TITLE Increased expression of a 58-kDa protein kinase leads to changes in the CHO cell cycle
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 87, 7467-7471 (1990)
MEDLINE 91017527
REFERENCE 2 (sites)
AUTHORS Bunnell,B.A., Heath,L.S., Adams,D.E., Lahti,J.M. and Kidd,V.J.
TITLE Corrections
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 88, 2612-2612 (1992)
MEDLINE 91172857

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ATAGGTGTTTGTT

LOCUS HS3OCOAT 1391 bp mRNA linear PRI 12-SEP-1993
DEFINITION Human mRNA for 3-oxoacyl-CoA peroxisomal thiolase.
ACCESSION X12966
VERSION X12966.1 GI:23873
KEYWORDS 3-oxoacyl-CoA thiolase; thiolase.
SOURCE human.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 867; 869 to 1288; 1290 to 1391)
AUTHORS Bout,A.
TITLE Direct Submission
JOURNAL Submitted (20-SEP-1988) Bout A., Dept of Biochemistry, University
of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam
REFERENCE 2 (bases 1 to 867; 869 to 1288; 1290 to 1391)
AUTHORS Bout,A., Teunissen,Y., Hashimoto,T., Benne,R. and Tager,J.M.
TITLE Nucleotide sequence of human peroxisomal 3-oxoacyl-CoA thiolase
JOURNAL Nucleic Acids Res. 16 (21), 10369 (1988)
MEDLINE 89057483

>gi|23873|emb|X12966.1|HS3OCOAT Human mRNA for 3-oxoacyl-CoA peroxisomal thiolase CDS 7..1281
TGCGC*AATGCAGAGGCTG*CAGGTAGTGCTGGGCCACCTGAGGGGTCCGGCCGATTCCGGCTGGATGCCGC
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LOCUS HUMSP10A 1043 bp mRNA linear PRI 03-AUG-1993
DEFINITION Human sperm protein 10 mRNA, complete cds.
ACCESSION M82967
VERSION M82967.1 GI:338291
KEYWORDS sperm protein 10.
SOURCE Homo sapiens male adult testis cDNA to mRNA.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1043)
AUTHORS Wright,R.M., John,E., Klotz,K., Flickinger,C.J. and Herr,J.C.
TITLE Cloning and sequencing of cDNAs coding for the human
intra-acrosomal antigen SP-10
JOURNAL Biol. Reprod. 42, 693-701 (1990)
MEDLINE 90268085

>gi|338291|gb|M82967.1|HUMSP10A Human sperm protein 10 mRNA, complete cds CDS 53..793
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GTCTTCCCTGCTCCAACTCTTTCTCTCAATATTCCTGATTTTTTTTTCAATAAATTTCACATG

LOCUS HUMMITCORA 1985 bp mRNA linear PRI 25-JUL-1994
DEFINITION Human ubiquinol cytochrome-c reductase core I protein mRNA, complete cds.
ACCESSION L16842
VERSION L16842.1 GI:349472
KEYWORDS core protein I; mitochondrial respiratory chain; ubiquinol cytochrome-c reductase.
SOURCE Homo sapiens cDNA to mRNA.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 1985)
AUTHORS Hoffman,G.G., Lee,S., Christiano,A.M., Chung-Honet,L.C., Cheng,W., Katchman,S., Uitto,J. and Greenspan,D.S.
TITLE Complete coding sequence, intron/exon organization, and chromosomal location of the gene for the core I protein of human ubiquinol-cytochrome c reductase
JOURNAL J. Biol. Chem. 268, 21113-21119 (1993)

>gi|349472|gb|L16842.1|HUMMITCORA Human ubiquinol cytochrome-c reductase core I protein mRNA, complete cds CDS 418..1860

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CCACCAATAAAATCCTCTGCTGAGA

US006080576A

United States Patent [19]

Zambrowicz et al.

[11] Patent Number: 6,080,576

[45] Date of Patent: Jun. 27, 2000

[54] **VECTORS FOR GENE TRAPPING AND GENE ACTIVATION**

[75] Inventors: **Brian Zambrowicz; Glenn Friedrich; Arthur T. Sands**, all of The Woodlands, Tex.

[73] Assignee: **Lexicon Genetics Incorporated**, The Woodlands, Tex.

[21] Appl. No.: **09/057,328**

[22] Filed: **Apr. 8, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/079,729, Mar. 27, 1998.

[51] **Int. Cl.**[7] **C12N 15/63**; C12N 15/85; C12N 15/00

[52] **U.S. Cl.** **435/320.1**; 435/325; 435/455; 435/463

[58] **Field of Search** 435/320.1, 325; 800/8, 13, 18

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Primary Examiner—Bruce R. Campell
Assistant Examiner—AnneMarie S. Beckerleg
Attorney, Agent, or Firm—Pennie & Edmonds LLP

[57] **ABSTRACT**

A novel 3' gene trap cassette is described that does not encode a marker conferring antibiotic resistance and can be used to efficiently trap and identify cellular genes. Vectors incorporating the presently 3' gene trap cassette find particular application in gene discovery, the production of transgenic cells and animals, and gene activation.

21 Claims, 1 Drawing Sheet

(e) a second retroviral LTR sequence;

wherein said promoter, exon and splice donor are present in the vector in between said first and second LTR sequence and in an opposite orientation to said first and second retroviral LTR sequences and wherein said vector does not incorporate a sequence that mediates the polyadenylation of an mRNA transcript expressed by said promoter of element (b) and encoded by said exon of element (c).

7. A method of gene trapping comprising introducing a vector according to any one of claims 1, 2 and 3 through 6 into an isolated eukaryotic target cell.

8. The method of claim 7 wherein said introducing of said vector into said eukaryotic target cell is carried out by a method drawn from the group consisting of electroporation, viral infection, retrotransposition, microinjection, and transfection.

9. The method of claim 8 wherein said eukaryotic cell is a mammalian cell.

10. The method of claim 9 wherein said mammalian cell is a murine embryonic stem cell.

11. A method of generating a library of isolated, nonspecifically mutated eukaryotic cells comprising introducing a vector according to any one of claims 1, 5 or 6 into eukaryotic cells to produce a collection of isolated, nonspecifically mutated eukaryotic cells.

12. A method to activate the expression of a naturally occurring gene in an isolated cell comprising introducing a vector according to any one of claims 1, 5 or 6 into said cell.

13. The method of claim 12 wherein said cell is a mammalian cell.

14. The method of claim 13 wherein said mammalian cell is a human cell.

15. A method to alter the expression of a gene in an isolated eukaryotic cell comprising introducing a 3' gene trap cassette vector into said cell, said 3' gene trap cassette comprising in operable combination:

1) a promoter;

2) an exon sequence located 3' from and expressed by said promoter, said exon being derived from a naturally occuring eukaryoric one said exon not encoding an activity conferring antibiotic resistance and said exon not being a reporter gene; and

3) a splice donor sequence defining the 3' region of said exon said slice donor sequence being derived from a naturally occuring eukaryotic gene;

wherein said cassette is non-homologously incorporated into the genome of an eukaryotic target cell and said splice donor sequence of the transcript encoded by said exon is spliced to a splice acceptor sequence of said cellularly encoded gene.

16. The method of claim 15 wherein said non-homologously incorporated cassette is present in a retroviral vector that has randomly integrated into the genome of the eukaryotic target cell.

17. The method of claim 16 wherein said eukaryotic target cell is an animal cell.

18. The method of claim 17 wherein said animal cell is a mammalian cell.

19. The method of claim 18 wherein said mammalian cell is a human cell.

20. The method of claim 18 wherein said mammalian cell is a rodent cell.

21. The method of claim 20 wherein said rodent cell is a mouse cell.

* * * * *

US005733761A

United States Patent [19]

Treco et al.

[11] Patent Number: 5,733,761

[45] Date of Patent: Mar. 31, 1998

[54] **PROTEIN PRODUCTION AND PROTEIN DELIVERY**

[75] Inventors: **Douglas Treco**, Arlington; **Michael W. Heartlein**, Boxborough; **Richard F. Selden**, Wellesley, all of Mass.

[73] Assignee: **Transkaryotic Therapies, Inc.**, Cambridge, Mass.

[21] Appl. No.: 451,893

[22] Filed: May 26, 1995

Related U.S. Application Data

[63] Continuation of Ser. No. 985,586, Dec. 3, 1992, abandoned, which is a continuation-in-part of Ser. No. 789,188, Nov. 5, 1991, abandoned, Ser. No. 911,533, Jul. 10, 1992, abandoned, and Ser. No. 787,840, Nov. 5, 1991, abandoned.

[51] Int. Cl.[6] C12N 15/00; C12P 21/00; C07H 21/04

[52] U.S. Cl. 435/172.3; 435/69.4; 536/23.51; 536/241

[58] Field of Search 435/172.3, 69.1, 435/69.4; 536/23.1, 23.51, 24.1

[56] **References Cited**

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89 01517	2/1989	WIPO .
90/11354	10/1990	WIPO .
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91 00361	1/1991	WIPO .
91/06666	5/1991	WIPO .
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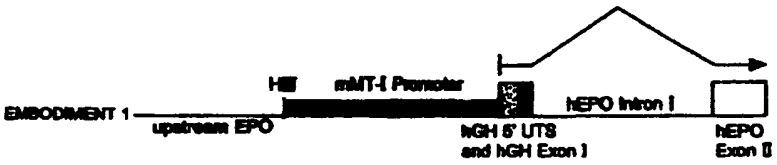
Primary Examiner—George C. Elliott
Assistant Examiner—Robert Schwartzman
Attorney, Agent, or Firm—Fish & Richardson P.C.

[57] **ABSTRACT**

The present invention relates to transfected primary, secondary and immortalized cells of vertebrate origin, particularly mammalian origin, transfected with exogenous genetic material (DNA) which encodes a desired (e.g., a therapeutic) product or is itself a desired (e.g., therapeutic) product, methods by which primary, secondary and immortalized cells are transfected to include exogenous genetic material, including DNA targeting by homologous recombination, methods for the activation and amplification of endogenous cellular genes, methods by which cells useful for large-scale protein production can be obtained, methods of producing clonal cell strains or heterogenous cell strains, and methods of gene therapy in which transfected primary, secondary or immortalized cells are used. The present invention includes primary, secondary and immortalized cells, such as fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, and other cells which can be cultured.

70 Claims, 5 Drawing Sheets



16. The DNA construct of claim 7 wherein the construct further comprises a second targeting sequence homologous to a sequence upstream of the coding region of the targeted gene.

17. The DNA construct of claim 7 wherein the construct further comprises a second targeting sequence homologous to a sequence upstream of the endogenous regulatory sequence of the targeted gene.

18. The DNA construct of claim 7 wherein the targeted gene encodes a therapeutic protein.

19. The DNA construct of claim 7 wherein the targeted gene encodes a hormone, a cytokine, an antigen, an antibody, an enzyme, a clotting factor, a transport protein, a receptor, a regulatory protein or a structural protein or a transcription protein.

20. The DNA construct of claim 7 wherein the targeted gene encodes a protein selected from the group consisting of erythropoietin, calcitonin, growth hormone, insulin, insulinotropin, insulin-like growth factors, parathyroid hormone, interferons, nerve growth factors, glucagon, interleukins, colony stimulating factors, immunoglobulins, catalytic antibodies, glucocerebrosidase, superoxide dismutase, tissue plasminogen activator, Factor VIII, Factor IX, apolipoprotein E, apolipoprotein A-I, globins, low density lipoprotein receptor, IL-2 receptor, IL-2 antagonists, alpha-1 antitrypsin and immune response modifiers.

21. The DNA construct of claim 9 further comprising one or more selectable markers.

22. The DNA construct of claim 20 wherein the targeted gene encodes growth hormone, insulinotropin, or a colony stimulating factor.

23. The DNA construct of claim 20 wherein the targeted gene encodes erythropoietin.

24. The DNA construct of claim 21 further comprising an amplifiable marker gene.

25. The DNA construct of claim 23 wherein the DNA of the exon encodes a protein fragment which is the same as the protein fragment encoded by the DNA of the first exon of erythropoietin.

26. The DNA construct of claim 23 wherein the DNA of the exon encodes a protein fragment which is different from the protein fragment encoded by the DNA of the first exon of erythropoietin.

27. The DNA construct of claim 23 wherein the DNA of the exon encodes a protein fragment which is the same as the protein fragment encoded by the DNA of the first exon of human growth hormone.

28. A method of making a homologously recombinant cell in vitro wherein the expression of a targeted gene not normally expressed in the cell is altered, comprising the steps of:

(a) transfecting the cell in vitro with a DNA construct, the DNA construct comprising:

(i) a targeting sequence;

(ii) a regulatory sequence;

(iii) an exon; and

(iv) an unpaired splice donor site, thereby producing a transfected cell; and

(b) maintaining the transfected cell in vitro under conditions appropriate for homologous recombination.

29. The method of claim 28 wherein the exon comprises a CAP site.

30. The method of claim 28 wherein the targeted gene encodes a protein selected from the group consisting of: erythropoietin and growth hormone.

31. The method of claim 29 wherein the exon comprises the nucleotide sequence ATG.

32. The method of claim 31 wherein the DNA encodes a protein fragment which is the same as the protein fragment encoded by the DNA of the first exon of erythropoietin.

33. The method of claim 30 wherein the DNA encodes a protein fragment which is different from the protein fragment encoded by the DNA of the first exon of erythropoietin.

34. The method of claim 30 wherein the DNA of the exon encodes a protein fragment which is the same as the protein fragment encoded by the DNA of the first exon of human growth hormone.

35. The method of claim 31 wherein the exon further comprises DNA which encodes a protein fragment and is in-frame with the targeted gene after homologous recombination with chromosomal DNA.

36. The method of claim 35 wherein the DNA of the exon encodes a protein fragment which is the same as the protein fragment encoded by the DNA of the first exon of the targeted gene.

37. The method of claim 35 wherein the DNA of the exon encodes a protein fragment which is different from the protein fragment encoded by the DNA of the first exon of the targeted gene.

38. The method of claim 35 wherein the targeting sequence is homologous to a sequence within the targeted gene.

39. The method of claim 35 wherein the targeting sequence is homologous to a sequence upstream of the coding region of the targeted gene.

40. The method of claim 35 wherein the targeting sequence is homologous to a sequence upstream of the endogenous regulatory sequence of the targeted gene.

41. The method of claim 35 wherein the construct further comprises a second targeting sequence homologous to a sequence within the targeted gene.

42. The method of claim 35 wherein the construct further comprises a second targeting sequence homologous to a sequence upstream of the coding region of the targeted gene.

43. The method of claim 35 wherein the construct further comprises a second targeting sequence homologous to a sequence upstream of the endogenous regulatory sequence of the targeted gene.

44. The method of claim 35 wherein the cell is a human cell.

45. A method of altering the expression of a targeted gene in a cell in vitro wherein the gene is not normally expressed in the cell, comprising the steps of:

(a) transfecting the cell in vitro with a DNA construct, the DNA construct comprising:

(i) a targeting sequence;

(ii) a regulatory sequence;

(iii) an exon; and

(iv) an unpaired splice donor site, thereby producing a transfected cell;and

(b) maintaining the transfected cell in vitro under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and

(c) maintaining the homologously recombinant cell in vitro under conditions appropriate for expression of the gene.

46. The method of claim 45 wherein the exon comprises the nucleotide sequence ATG.

47. The method of claim 46 wherein the exon further comprises a CAP site.

48. The method of claim 47 wherein the exon further comprises DNA which encodes a protein fragment and is in-frame with the targeted gene after homologous recombination.